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The ATPase Inhibitory Factor 1 (IF1): A master regulator of energy metabolism and of cell survival

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SUMMARY

In this contribution we summarize most of the findings reported for the molecular and cellular biology of the physiological inhibitor of the mitochondrial H^+-ATP synthase, the engine of oxidative phosphorylation (OXPHOS) and gate of cell death. We first describe the structure and major mechanisms and molecules that regulate the activity of the ATP synthase placing the ATPase Inhibitory Factor 1 (IF1) as a major determinant in the regulation of the activity of the ATP synthase and hence of OXPHOS. Next, we summarize the post-transcriptional mechanisms that regulate the expression of IF1 and emphasize, in addition to the regulation afforded by the protonation state of histidine residues, that the activity of IF1 as an inhibitor of the ATP synthase is also regulated by phosphorylation of a serine residue. Phosphorylation of S39 in IF1 by the action of a mitochondrial cAMP-dependent protein kinase A hampers its interaction with the ATP synthase, *i.e.*, only dephosphorylated IF1 interacts with the enzyme. Upon IF1 interaction with the ATP synthase both the synthetic and hydrolytic activities of the engine of OXPHOS are inhibited. These findings are further placed into the physiological context to stress the emerging roles played by IF1 in metabolic reprogramming in cancer, in hypoxia and in cellular differentiation. We review also the implication of IF1 in other cellular situations that involve the malfunctioning of mitochondria. Special emphasis is given to the role of IF1 as driver of the generation of a reactive oxygen species signal that, emanating from mitochondria, is able to reprogram the nucleus of the cell to confer by various signaling pathways a cell-death resistant phenotype against oxidative stress. Overall, our intention is to highlight the urgent need of further investigations in the molecular and cellular biology of IF1 and of its target, the ATP synthase, to unveil new therapeutic strategies in human pathology.

1. Introduction

Mitochondria are highly dynamic double-membrane organelles of the eukaryotic cell that show a great variability in number, structure and function depending on the cell type and stage of development considered [1–6]. Mitochondria interact with elements of the cytoskeleton, for their subcellular localization and motility [7,8] and with the endoplasmic reticulum [9], through ERMES protein complexes [10], which are involved in calcium homeostasis and lipid biogenesis. Mitochondria are essential for the provision of metabolic energy by oxidative phosphorylation (OXPHOS) in differentiated aerobic cells [11–13], for the execution of cell death [14,15] and for intracellular redox and calcium signaling [15,16] among other functions.

The outer mitochondrial membrane (OMM) has a specific complement of proteins and is freely permeable to protons. In contrast, the inner mitochondrial membrane (IMM), where OXPHOS takes place, has a protein and lipid composition that varies greatly from the OMM, it is impermeable to protons and it is extensively compartmentalized by self-invaginations called *cristae* (Fig. 1A). In OXPHOS, the electrons obtained in biological oxidations are funneled into the protein complexes of the electron transport chain (ETC) (Fig. 1B). During electron transfer complexes I, III and IV pump protons out of the mitochondrial matrix to the intermembrane space to generate the proton electrochemical gradient that is used as driving force for the import of different metabolites and for the synthesis of ATP by the ATP synthase [17] (Fig. 1B).

Cristae of the IMM are bound to the OMM by inner membrane peripheral areas known as *cristae* junctions. These contacts depend on the mitochondrial contact site and cristae organizing system (MICOS) [18]. MICOS is composed by mitochondrial membrane proteins that upon oligomerization are able to bend the mitochondrial membrane [19]. *Cristae* locate all complexes of the ETC and thus increase the total surface of the IMM to generate more functional respiratory surface. The biogenesis and remodeling of mitochondrial *cristae* depend on multiple factors [20], being the most relevant one the ATP synthase. Specifically, formation of ATP synthase dimers in a V-shaped structure with an angle of 86° between monomers generates the curvature on the IMM that promotes *cristae* formation [21,22] (Fig. 1A). Interestingly, aging

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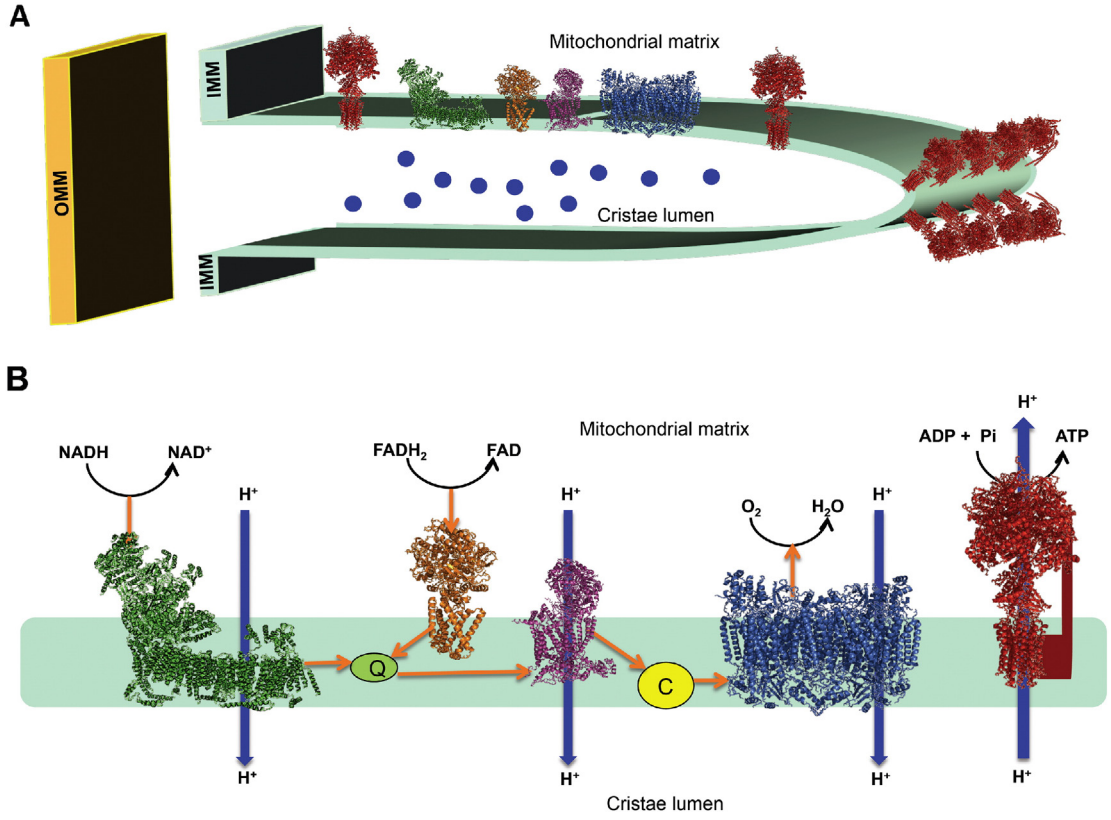

Fig. 1. Assembly of OXPHOS complexes in the inner mitochondrial membrane. A, Invaginations of the inner membrane (IMM, light green) generate mitochondrial *cristae*. Dimers of the ATP synthase (red) promote the curvature of the IMM in the edge of *cristae*. Protein complexes of the electron transport chain (green, purple and blue) pump protons (blue spheres) into the *cristae* lumen during respiration. Outer mitochondrial membrane (OMM, orange) delimits the intermembrane space. B, Zoom of the mitochondrial *cristae*. The transfer of electrons (orange arrows) and proton pumping activity of respiratory complexes (blue arrows) are indicated. In A–B, images of the crystallographic structure of the complexes are taken with PyMOL Molecular Graphics System. Complex I (green) (PDB: 4QU8) [252], complex II (orange) (PDB: 1ZOY) [253], complex III (purple) (PDB: 1BE3) [254] and complex IV (blue) (PDB: 1OCC) [255]. The ATP synthase F1-domain and c-ring (red) in monomeric form (PDB: 2XND) [256] and the dimer in yeast (PDB: 4B2Q) [21]. Q: coenzyme Q (green sphere). C: cytochrome c (yellow sphere).

seems to melt down mitochondrial ultrastructure by age-dependent dissociation of ATP synthase dimers [23].

Recent findings support that respiratory complexes are assembled in higher order structures named supercomplexes or respirasomes [24] to optimize electron transfer and minimize the production of reactive oxygen species (ROS). Evidence for the existence of functional respirasomes that are able to transfer electrons from NADH to oxygen [25] and to respond to the variable energy [26] or structural [27] requirements of the cell has been provided.

2. The mitochondrial ATP synthase: structure and assembly

The mitochondrial ATP synthase is a protein complex built by two main domains [28]. The F1-ATPase domain is extrinsic to the IMM and is composed of 3α, 3β and 1γ subunit. The Fo-ATPase domain is embedded in the membrane and is composed by a ring of 8–10 c subunits and 1a subunit. Both domains are linked together by a peripheral arm composed by different subunits (1b/1F6/1d/1A6L/1OSCP) and the central arm that, in addition to the γ subunit of the F1-ATPase domain, contains one δ and ε subunits. There are several additional subunits that contact and span the Fo domain by its peripheral and central stalk (1A6L/1e/1f/1g). The 3α and 3β subunits in the F1 domain form a spherical alternate structure enveloping the extended α-helix conformation of the γ subunit, which constitutes the central stalk of the structure. The rest of the γ subunit is tightly bound to the Fo-ATPase domain through subunits δ and ε. The Fo-ATPase domain is a hydrophobic cylindrical structure consisting of a ring of c subunits that along with subunits γ, δ and ε form the rotor of the engine [28].

The catalytic activity of the H^+-ATP synthase is based on the asymmetric assembly of the F1-ATPase domain due to the disposition of the γ subunit that forces each one of the three catalytic β subunits to adopt a different conformation with variable nucleotide binding and catalytic properties [28]. A key to catalysis are the conformational changes experienced by the β subunits due to the rotation of the γ subunit. Through 360° rotation of the γ subunit the ATP synthase synthesizes three molecules of ATP. Since the ATP synthase is a reversible rotatory engine [29] it can hydrolyze three molecules of ATP when functioning in reverse. ATP synthesis needs the proton electrochemical gradient as driving force to power the rotation of the cylinder in the Fo-domain while, when functioning in reverse, ATP hydrolysis pumps H^+ out to the intermembrane space.

The assembly of the H^+-ATP synthase is a partially characterized process [28,30,31]. In *Saccharomyces cerevisiae* chaperones for the assembly of the F1-ATPase [32] and the Fo-ATPase domains have been identified [31]. Late steps in the assembly of the mitochondrial ATP synthase are mediated by the inner membrane assembly complex INA [33]. The supernumerary subunits e and g are known to play a role in promoting dimerization of the ATP synthase [28,31]. Subunit b in the peripheral arm is also a major determinant in maintaining oligomeric structures of the ATP synthase [34]. Interestingly, the dimeric structure of ATP synthase, independently of its function in oxidative phosphorylation, has been shown to play a central role in the differentiation of germinal lines of ovarian stem cells in *Drosophila melanogaster* by promoting the maturation of *cristae* [35]. Other proteins described with a role in maintaining a correct assembly of the enzyme are the mitochondrial fusion protein OPA1 [36], LRPPRC that when inactivated generates sub-assemblies of the ATP synthase [37] and cyclophilin D

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